RESEARCH ARTICLE

RAPD and microsatellite transferability studies in selected species of *Prosopis* (section Algarobia) with emphasis on *Prosopis juliflora* and *P. pallida*

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Abstract

The genus *Prosopis* (Leguminosae, Mimosoideae), comprises 44 species widely distributed in arid and semi-arid zones. *Prosopis pallida* (Humb. & Bonpl. ex Willd.) Kunth and *P. juliflora* (Sw.) DC. are the two species that are truly tropical apart from *P. africana*, which is native to tropical Africa (Pasiecznik *et al.* 2004), and they have been introduced widely beyond their native ranges. However, taxonomic confusion within the genus has hampered exploitation and better management of the species. The present study focusses primarily on evaluating the genetic relationship between *Prosopis* species from the section Algarobia, containing most species of economic importance, though *P. tamarugo* from section Strombocarpa is also included for comparison. In total, 12 *Prosopis* species and a putative *P. pallida* × *P. chilensis* hybrid were assessed for their genetic relationships based on RAPD markers and microsatellite transferability. The results show that *P. pallida* and *P. juliflora* are not closely related despite some morphological similarity. Evidence also agrees with previous studies which suggest that the grouping of series in section Algarobia is artificial.

[Sherry M., Smith S., Patel A., Harris P., Hand P., Trenchard L. and Henderson J. 2011 RAPD and microsatellite transferability studies in selected species of *Prosopis* (section Algarobia) with emphasis on *Prosopis juliflora* and *P. pallida*. *J. Genet.* **90**, 251–264]

Introduction

The genus *Prosopis* Linnaeus emend. Burkart, belongs to the family Leguminosae, subfamily Mimosoideae, tribe Mimosae. Burkart (1976) identified 44 species in the genus *Prosopis*, the taxonomic classification being based on morphological differences between *Prosopis* specimens. Some of the species in the genus are of major economic importance, primarily their pods are utilized as food and fodder, and wood as fuel and timber (Pasiecznik *et al*. 2001). Their ability to thrive in extreme environmental conditions and provision of food, fodder, fuel and timber encouraged their introduction to economically poor regions of the world (Hughes 1994). Most of the species are native to the Americas, with one from tropical Africa and three from southwestern Asia and northeastern Africa (Burkart 1976; Hunziker *et al*. 1986). Burkart (1976) divided the genus *Prosopis* into five sections based on observed morphological differences, two from Africa and Asia, with species from three of these sections, Algarobia, Monilicarpa and Strombocarpa occupying the Americas.

The section Algarobia comprises the largest number of species, including 31 of varying habit. Burkart (1976) further divided this section into six series, namely, Sericanthae, Ruscifoliae, Humiles, Denudantes, Pallidae and Chilensis. The section Strombocarpa containing eight species and is divided into two series Strombocarpae and Cavenicarpae, and section Monilicarpa is monospecific. Although similarities exist in terms of floral and leaf morphologies between species from the former two sections, Burkart (1976) identified certain distinct morphological characters separating the two. The coiling pattern of the fruit and spine development is clearly

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Keywords. RAPD; microsatellites; genetic relationship; *Prosopis juliflora*; *P. pallida*.

distinguishable and separates species of section Algarobia from section Strombocarpa (Burkart 1976). Species of section Algarobia are mostly selfincompatible and outcross (Solbrig and Cantino 1975; Simpson 1977), although a small percentage of selfing is reported (Bessega *et al.* 2000). Enzyme and molecular marker studies have concluded that section Algarobia is not a natural group (Bessega *et al.* 2005; Burghardt and Espert 2007). Although isozyme analysis proved to be useful, the enzymatic expression is restricted to specific developmental stages or tissues influenced by the environment (Solbrig and Bawa 1975; Whitmore and Bragg 1979; Saidman and Vilardi 1987). However, RAPD markers have shown clear advantages over isozymes in detecting genetic variability between species within the genus (Goswami and Ranade 1999; Bessega *et al.* 2000; Ferreyra *et al.* 2007).

Prosopis pallida (Humb. & Bonpl. ex Willd.) Kunth and *P. juliflora* (Sw.) DC., two truly tropical species of this genus (Pasiecznik *et al.* 2001), belong to section Algarobia. The possibility of interspecific hybridization among species of this section has been reported by Naranjo *et al.* (1984) and Hunziker *et al.* (1986). The two species are similar in leaf morphology and tree form, and Pasiecznik *et al.* (2001) assumed that naturally occurring hybridization in their overlapping native ranges led to the difficulty in differentiating the two species. Molecular studies were useful in differentiating the two species and reported them to be closely related (Landeras *et al.* 2006), and this and another study (Harris *et al.* 2003) confirmed that *P. juliflora* was the only known naturally occurring tetraploid species in the genus, though the origin of *P. juliflora* remained unresolved. Further molecular analysis was noted to be essential in order to fully exploit these species especially in the areas where they are introduced. Molecular differentiation of the two species would help in their better management and genetic improvement.

In the present work, RAPD and microsatellite primer pairs were utilized to assess the genetic relationship and cross species amplification profile of selected *Prosopis* species. Through RAPD analysis, the genetic relationship and clustering pattern of eight species of *Prosopis* were analysed, namely *Prosopis alba, P. chilensis, P. pallida, P. juliflora, P. glandulosa* var. *torreyana, P. laevigata, P. articulata* and *P. velutina* from section Algarobia and *P. tamarugo* from section Strombocarpa. Except for *P. pallida,* all the other Alagrobia species selected are from series Chilensis; *P. pallida* being from series Pallidae. Further, a total of six microsatellite primer pairs designed for *Prosopis chilensis* and *Prosopis flexuosa* by Mottura *et al.* (2005) were also screened for possible transferability and amplification. Three more species, *P. nigra, P. caldenia* and *P. affinis* all from section Algarobia were also selected for the microsatellite transferability studies. The high rate of transfer of markers among related species would suggest homology in the DNA sequences in SSR flanking regions (Primmer and Merila 2002). These SSR flanking sequences are conserved and could be used to examine evolutionary relationships (Rosseto *et al.* 2002).

Materials and methods

Prosopis accessions

Eleven out of the 12 *Prosopis* species selected for RAPD and microsatellite cross species amplification study represent the section Algarobia. The only selected species from Strombocarpa, *P. tamarugo* is from the series Cavenicarpae of that section. For the RAPD analysis, only nine species representing the two sections were selected. Eight of them were from the two series, Pallidae and Chilensis of the section Algarobia. The seeds of all the 11 species of *Prosopis*, except for *P. juliflora*, analysed in this study were obtained from the Henry Doubleday Research Association (HDRA, Coventry, UK), UK and Danida Forest Seed Centre (DFSC, Humlebaek, Denmark), Denmark. These were collected from a single tree or from several trees in a population either from their natural or introduced ranges. *P. tamarugo* seeds from Strombocarpa were selected from its native range. Seeds of the Algarobia species *P. chilensis, P. pallida, P. laevigata, P. glandulosa* var. *torreyana, P. caldenia* and *P. nigra* were also selected from their native ranges (Johnston 1962; Burkart 1976). Seeds of *P. alba, P. articulata* and *P. velutina* were collected from California, USA.

The seeds of *P. juliflora* were obtained from the introduced range in the Galapagos Islands and seedlings were established at the Coventry University greenhouse. The identity of *P. juliflora* samples from Galapagos was initially established using leaf morphology before its ploidy confirmation by flow cytometry (Trenchard *et al.* 2008). *P. juliflora* populations in the Galapagos Islands might have become naturalized through numerous introductions (Wiggins and Porter 1971). For the RAPD and microsatellite study, the selected species from Danida Forest Seed Centre (DFSC) were identified by collectors, by comparing the species morphology with botanical descriptions (Lars Schmidt, Seed Biologist, DFSC, Humlebaek, Denmark). The species identity for the seeds obtained from HDRA was taxonomically determined by Dr Peter Felker (Research Scientist, D'Arrigo Bros, California, USA). Seeds of a putative *P. chilensis* × *P. pallida* hybrid from Cape Verde were also obtained from HDRA and are included in the present study. The hybrid was putatively identified by the collector Nick Pasiecznik based on the mother tree and the fact that all surrounding flowering trees were of a single different species. Genomic DNA was extracted from leaves in case of *P. juliflora* and from the cotyledons of the other species. A collective list of the *Prosopis* species, their taxonomic information and sources are given in table 1.

DNA extraction

Bulked DNA samples were prepared for extraction as described by Michelmore *et al.* (1991). From each species 10 to 15 seeds were randomly selected for RAPD analysis. The seeds were first rinsed in ordinary tap water for 1 or 2 min and then in de-ionised water for at least 2 min.

Table 1. List of the *Prosopis* sections, series and species studied with their accession number, country of origin and source.

Section	Series	*Prosopis* species	Accession number	Provenance/ country	Collector/source (if known)
Strombocarpa	Cavenicarpae	*P. tamarugo*	HDRA 562	1019/Chile	P. Felker/HDRA
Algarobia	Pallidae	*P. pallida*	DAN 1490/85	San Jacinto De Cachche/Peru	DFSC
Algarobia	Pallidae	*P. affinis*	DAN 1653/86	Codigo C1/Peru	DFSC
Algarobia	Pallidae	*P. articulata*	HDRA 349	0593/USA	P. Felker/HDRA
–	–	*P. chilensis* × *P. pallida* hybrid?	HDRA 813	Monte Vaca/Cape Verde	N. Pasiecznik/HDRA
Algarobia	Chilensis	*P. chilensis*	HDRA 314.4	Chile	University of Chile/HDRA
Algarobia	Chilensis	*P. juliflora*	Gal 01	Galapagos Islands	Coventry University
Algarobia	Chilensis	*P. nigra*	HDRA 568	1135/Argentina	P. Felker/HDRA
Algarobia	Chilensis	*P. caldenia*	HDRA 652	AH542/90/Argentina	University of Cordoba/HDRA
Algarobia	Chilensis	*P. laevigata*	HDRA 810	OFI78/93/1/Mexico	J. Hawkins/HDRA
Algarobia	Chilensis	*P. glandulosa* var. *torreyana*	DAN 1211/83	Mexico	DFSC
Algarobia	Chilensis	*P. alba*	HDRA 905.5	0591/USA	P. Felker/HDRA
Algarobia	Chilensis	*P. velutina*	HDRA 545	0860/USA	P. Felker/HDRA

The seeds were then soaked in water containing two drops of nonionic surfactant viz. Nonidet P40 and shaken vigorously for few minutes. Then they were washed thoroughly with sterile distilled water until all the traces of surfactant were removed. The seeds were soaked for 10 min in 1 gL^{-1} of sodium dichloroisocyanurate (SDIC) and washed thoroughly with sterile distilled water before scarifying the testas mechanically to improve imbibition. The scarified seeds were then sown in Petri dishes under aseptic conditions and incubated overnight at 37°C. After incubation, the seeds were transferred to new Petri dishes lined with filter paper dampened with sterile distilled water. The Petri dishes were sealed with parafilm and germinated for 5 to 8 days at room temperature under 12:12 h LD and 80% relative humidity. When the cotyledons were fully expanded they were harvested for DNA extraction.

Total cellular DNA was extracted from 5-day-old cotyledons using the DNeasy Plant Mini kit (Qiagen, Crawley, UK) following the manufacturer's instructions. For each *Prosopis* species, cotyledons from 10 seedlings were bulked prior to extraction. About 30 μg of total cellular DNA was obtained using the kit. DNA concentrations were measured using NanoDrop® ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, USA) and the concentrations of total samples ranged from 20–90 ngμL^{-1}.

Randomly amplified polymorphic DNA (RAPD) analysis

Polymerase chain reaction: A total of 63 arbitrary primers were used and all were 10-mers custom synthesized by Invitrogen, Paisley, UK. Of these primers, only those which gave consistently good polymorphism and reproducibility were chosen for further experiments. List of 25 selected primers and their sequences are provided in table 2.

Polymerase chain reactions (PCR) for the generation of RAPD markers were performed with Go Taq Green master mix (Promega, Southampton, UK), 40–120 ng of genomic DNA as the template and 0.8 μM primer in a final volume of 25 μL. The mixture was then mixed gently and two drops of mineral oil were dropped on top of the mixture to prevent evaporation during the amplification cycles. The amplification was performed on a Peltier Thermal Cycler-200 (MJ Research, Waltham, USA). For each PCR, the following sequence was used: initial denaturation at 95°C for 2 min

Table 2. List of primers and their sequence used for phylogenetic study of 17 species of *Prosopis*.

Primers	Sequence
A08	5′- ACG CAC AAC C- 3′
B11	5′- GTA GAC CCG T- 3′
B12	5′- CCT TGA CGC A- 3′
E20	5′- ATC GGT GAC C- 3′
L01	5′- GGC ATG ACC T- 3′
L03	5′- CCA GCA GCT T- 3′
L09A	5′- AGC AGG TGG A- 3′
N03	5′- GGT ACT CCC C- 3′
N12	5′- CAC AGA CAC C- 3′
N14	5′- TCG TGC GGG T- 3′
R05	5′- TGC GCC CTT C- 3′
R07	5′- GGT GAC GCA G- 3′
R08	5′- GTC CAC ACG G- 3′
R18	5′- CCA CAG CAG T- 3′
R23	5′- AGC CAG GCT G- 3′
R25	5′- GGG TGC AGT T- 3′
R27	5′- TCG CTG CGG A- 3′
R29	5′- GGG GGA GAT G- 3′
R30	5′- CTG TGT GCT C- 3′
R32	5′- AAC GGC GGT C- 3′
R35	5′- TGA TGC CGC T- 3′
R37	5′- TCA GCA CAG G- 3′
R39	5′- ACC ACG CCT T- 3′
S07	5′- TCC GAT GCT G- 3′
S11	5′- AGT CGG GTG G- 3′

followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 42°C for 1 min and extension at 72°C for 1 min. The final primer extension was done at 72°C for 5 min.

Experiments were duplicated at different times with the same DNA and primers to ensure reproducibility. Two different controls, one without the template DNA and other without primer, were routinely used in all the experiments to rule out the possibility of any contamination.

Visualization of amplified DNA

The amplification products were separated by electrophoresis using a 1.5% agarose gel stained with ethidium bromide, EtBr (0.5 μgmL^{-1} for 30 min). It was visualized under the UV on a Biorad gel documentation system (Biorad, Hercules, USA). Images of the gels were copied and printed for the comparison of banding patterns.

Statistical analysis of data

Similarity index measurement: The different RAPD bands from the images were scored manually based on their presence (1) or absence (0) for each template–primer combination. Very faint bands were not scored. The binary data matrix was analysed using Dice's (1945) coefficient similarity to generate similarity coefficients between all possible pairs of 10 accessions of *Prosopis* including the hybrid. Similarity coefficients/indices measure the degree of relatedness between species and they can be applied to both qualitative (presence or absence data) and quantitative data (Kent and Coker 1992). The Dice coefficient (D) calculates similarity as $S_{ij} = (2a)/(2a + b + c)$, where S_{ij} is the similarity between two individuals *i* and *j*, a is the number of bands present in both *i* and *j*, b is the number of bands present in *i* and absent in *j* and c is number of bands present in *j* and absent in *i*. Dice's index is particularly useful for presence/absence and its value varies from zero (no similarity) to one (complete similarity). Hence the complementary genetic distance between accessions can be estimated by subtracting the similarity indices from 1 (Manly 2005).

Cluster analysis method

The main aim of this analysis was to find possible relations between the different *Prosopis* species according to their similarities. Species related closely are grouped in the same cluster and those dissimilar are grouped in separate clusters. This method increases the homogeneity between species in a cluster (Hair *et al*. 2006). A hierarchical clustering method clusters the species in several steps. Thus, different clusters are sequentially combined so that the number of clusters get reduced until a single cluster is achieved (Everitt and Dunn 2001). The hierarchical cluster method produces a dendogram which summarizes the entire taxonomy of all the species (Lessig 1972).

Several hierarchical clustering methods are available for phylogenetic analysis. For the present analysis, average linkage clustering algorithm (ALCA) or the group average method by Sokal and Michener (1958) was used. This is an agglomerative hierarchical clustering technique (Everitt and Dunn 2001). The similarity index calculated from Dice's coefficient was clustered with the group average method, where the dissimilarity between the clusters is determined by average distance between all possible clusters. The quality of this cluster analysis approach was tested by calculating the cophenetic correlation (CC). The CC coefficient finds the distortion between the similarity matrix and the dendogram (Romesberg 1984). A CC coefficient lower than 0.7 indicates that there has been distortion in the clustering process. This would prove the inadequacy of the clustering method to solve the data presented in the original similarity matrix. A CC value of 0.9 shows a sufficiently good correlation between the data matrix and dendogram (Mantel 1967; Romesberg 2004).

Microsatellite marker amplification

Microsatellite primer pairs: Six microsatellite loci developed for *P. chilensis* and *P. flexuosa* by Mottura *et al.* (2005) were used for the present amplification study. The reproducibility of amplification products were tested with two different

Table 3. Sequences and annealing temperature of six microsatellite loci (Mottura *et al*. 2005).

Locus	Primer sequences (5′-3′)	Annealing temperature (T_a)
Mo05	F: AATTCTGCAGTCTCTTCGCC R: GATCCCTCGTGACTCCTCAG	64°C
Mo07	F: GAAGCTCCCTCACATTTTGC R: CTATTTGCGCAACACACAGC	59°C
Mo08	F: TATCCTAAACGCCGGGCTAC R: TCCCATTCATGCATACTTAAACC	59°C
Mo09	F: ATTCCTCCCTCACATTTTGC R: CATTATGCCAGCCTTTGTTG	59°C
Mo13	F: TTGATTAGAGTTGCATGTGGATG R: TGCAGTCCCAAGTGTCAGAG	58°C
Mo16	F: CATTGCCCCAATATCACTCC R: GGGTCCATCCAGAGTAGTGG	60°C

DNA extractions of each species. The extractions were done on different days so that chances of cross contamination are avoided. PCR reactions on separate days confirmed the robustness of all the microsatellite markers. These primer pairs are highly variable producing well scorable and reproducible bands. Their sequence details and specific annealing temperature used in the PCR is summarized in table 3.

DNA amplification

PCRs were performed with 12.5 μL of 2× Go Taq Green master mix (Promega), 40–120 ng of genomic DNA as the template and 0.6 μM primer in a final volume of 25 μL. The mixture was then mixed gently and two drops of mineral oil were placed on top of the mixture to prevent evaporation during the amplification cycles. The amplification was performed using a Peltier Thermal Cycler-200. The cycling profile is as follows: an initial denaturation step of 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 45 s, a primer specific annealing step at 58–64°C for 45 s and an extension at 72°C for 45 s.

Resolution of amplification products

The amplified microsatellite markers were resolved by polyacrylamide gel electrophoresis (PAGE) for better separation of bands. PAGE was performed in 6% polyacrylamide gels without urea. The gel was prepared by diluting 30% acrylamide (29% w/v acrylamide and 1% w/v bisacrylamide) with 5× TBE (0.45 M Tris-borate, 0.01 M EDTA, pH 8.3 in molecular biology grade water), polymerized with 0.1% TEMED (N, N, N', N'-tetramethylethyleneamide) and 0.1% w/v ammonium per sulphate (freshly prepared 10% solution). The gel was run in 1× TBE at a constant voltage of 150 V for 1 h.

Analysis of amplified products

After electrophoresis, the gels were stained with ethidium bromide, EtBr (0.5 μgmL^{-1}) for 15 min. It was visualized under the UV on a Biorad gel documentation system. Images of the gels were copied and printed for marker analysis. The molecular sizes of amplified products were estimated using the 50-bp DNA step ladder (Promega) which consists of 16 DNA fragments of size ranging from 50 bp to 800 bp. Percentage of transferability of the microsatellite primer pairs were calculated using the formula, percentage transferability = (no. of species in which amplification occurred / total no. of species) × 100.

Results

RAPD analysis of Prosopis species

Primer selection: Randomly amplified polymorphic DNA (RAPD) patterns of nine *Prosopis* species and a hybrid were analysed for their identification and differentiation. Of the 63 arbitrary primers, 25 produced scorable, distinguishable bands and they were selected for subsequent experiments; others which produced less significant or faint bands were excluded. The 25 oligonucleotide primers yielded different polymorphic banding patterns that were unique to each primer and distinguishable over all species analysed. A total of 968 bands were generated by the primers in all the species analysed. The size of the observed markers ranged from 1500 bp to 140 bp.

RAPD markers

The *Prosopis* species selected for the study are mostly from the section Algarobia with the exception of *P. tamarugo* which is from section Strombocarpa. Few markers of taxonomic interest were amplified by the RAPD primers. The primer A08 generated three molecular markers of approximately 1000 bp, 800 bp and 290 bp which are present in all species of the section Algarobia. This marker is absent in *P. tamarugo*. Similarly, a fragment of approximate size 350 bp is amplified in all species except *P. tamarugo* belonging to section Strombocarpa using primer R18. The RAPD profile of primers A08 and R18 is shown in figures 1, a&b. The amplified markers and their size in base pairs can also be seen (arrows shown).

A few markers of interest are amplified in the North American species of *Prosopis*. The primer R37 generates a band of size 300 bp (figure 2a) which is commonly present in the central American *P. juliflora* and North American species. When the primer S07 was used, another marker of approximate size 550 bp (figure 2b) was amplified in all the North American species and *P. juliflora*. This primer also amplifies a fragment of approximate size 1100 bp in all the species of section Algarobia (figure 2b). Interestingly, primer R07 also yields a marker of size 1400 bp (figure 3a) which is present in the three North American Algarobia species.

Few unique banding profiles were generated in *P. pallida* and the putative *P. pallida* × *P. chilensis* hybrid. A molecular marker of approximately 380 bp is seen in the profile produced by primer R07 which is present in *P. pallida* and hybrid (figure 3a). The primer S11 also yields a 460 bp marker identifying *P. pallida* and the hybrid (figure 3b). RAPD primer N14 amplified fragments of size 1300 bp approximately in *P. pallida* and the hybrid alone (figure 4a). This fragment is not present in *P. chilensis*. The marker of size 1500 bp (arrow shown) generated by primer R08 (figure 4b) is only present in the hybrid and *P. pallida*, one of the putative parents of the hybrid.

RAPD primer N03 amplified fragments of size 710 bp and 550 bp approximately in *P. tamarugo* (figure 5a). Those bands are unique to the species and are not present in any Algarobia species. A 900-bp marker can also be seen amplified in *P. pallida* and the hybrid which is absent in other *Prosopis* species (figure 5a). When amplified with primer R05 there is a distinctive band of approximately 900 bp in

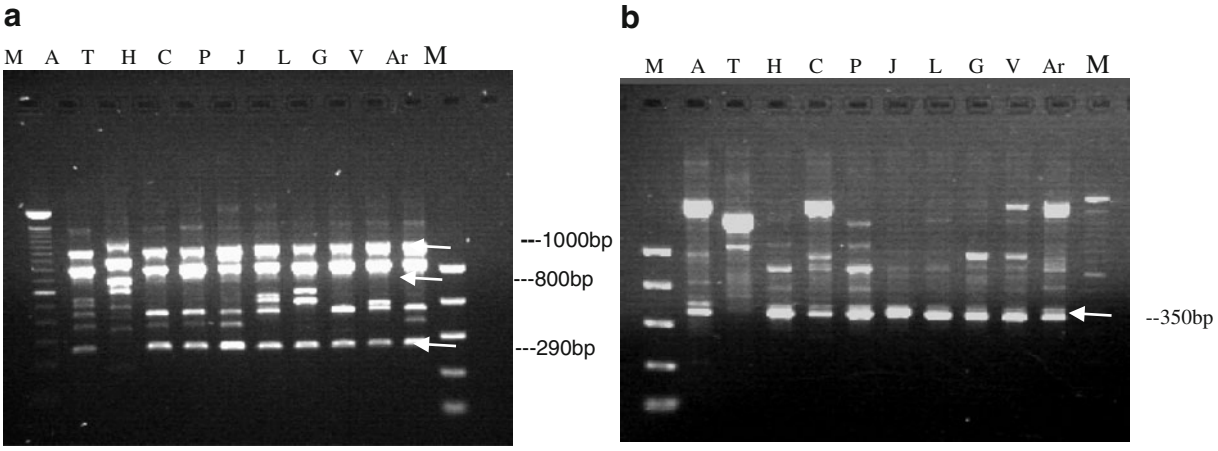

Figure 1. a, RAPD profile of primer A08 and b, primer R18. Lane names: M, molecular size marker, A, *P. alba*; T, *P. tamarugo*; H, *P. pallida* × *P. chilensis*?, C, *P. chilensis*; P, *P. pallida*; J, *P. juliflora*; L, *P. laevigata*; G, *P. glandulosa* var. *torreyana*; V, *P. velutina*; Ar, *P. articulata.*

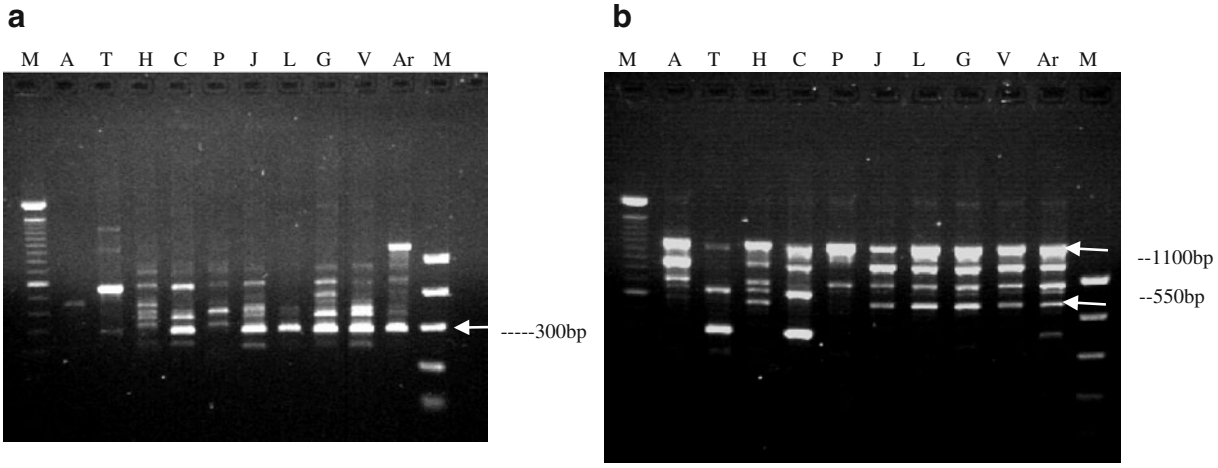

Figure 2. a, RAPD profile of primer R37 and b, S07. For lane names, see footnote of figure 1.

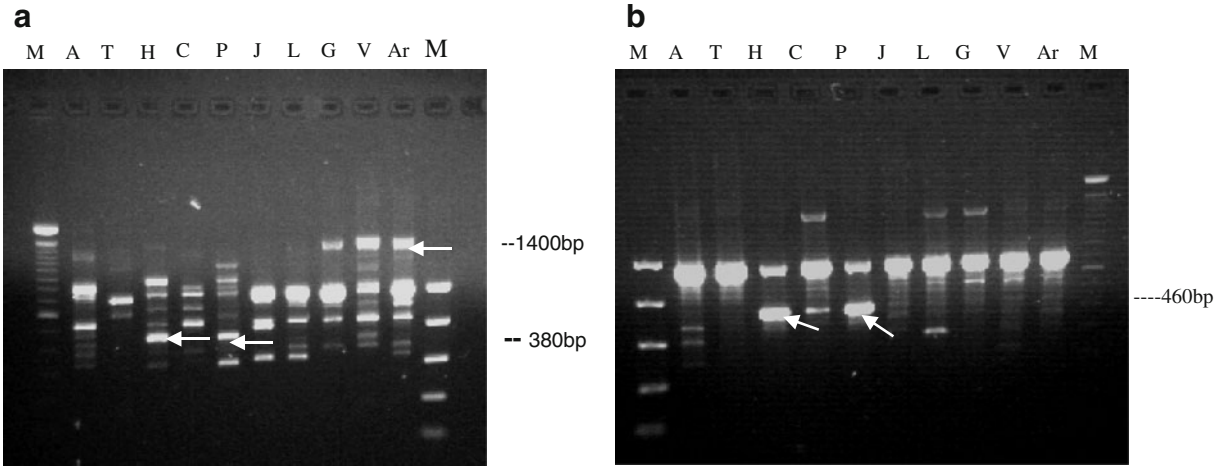

Figure 3. a, RAPD profile of primer R07 and b, S11.

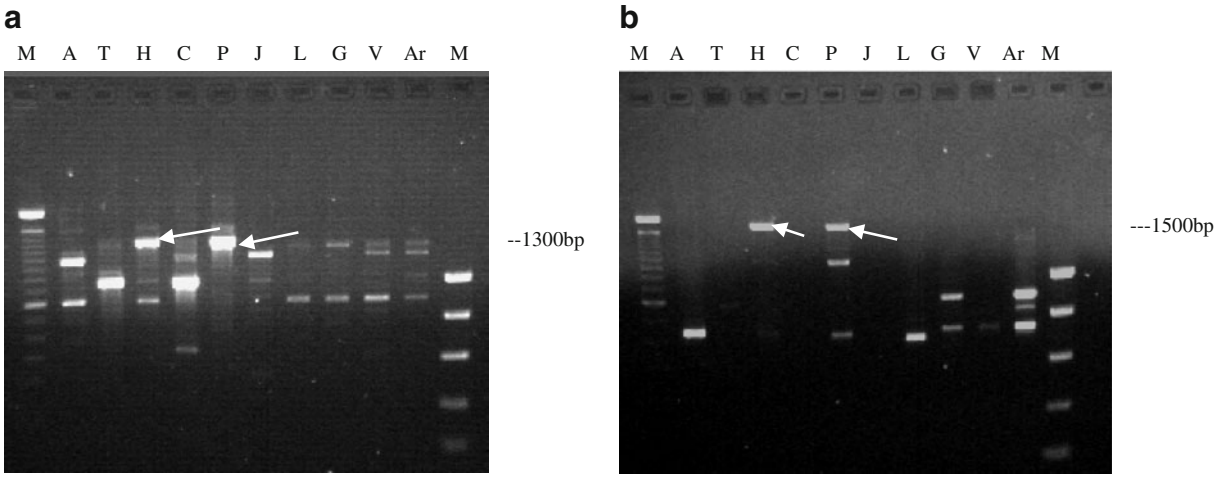

Figure 4. a, RAPD profile of primer N14 and b, R08.

size (arrow shown) present in *P. juliflora* which cannot be seen in any other species (figure 5b).

Cluster analysis

The main aim of the cluster analysis was to find the genetic relationship between the tested species of *Prosopis* belonging to section Algarobia. *P. tamarugo* from section Strombocarpa was also selected for comparison between the two sections. The relationship between tetraploid *P. juliflora* and diploid *P. pallida* was analysed closely since the origin of *P. juliflora* has yet to be identified. The group average dendogram shown in figure 6 identifies three major clusters.

Prosopis glandulosa and *P. laevigata* with *P. articulata* and *P. velutina* forms the two major clusters. The third major cluster involves *P. pallida* and the putative *P. pallida* × *P. chilensis* hybrid. The fourth cluster involves *P. chilensis* and *P. alba*. Two clusters formed by *P. articulata/P. velutina* and *P. glandulosa/P. laevigata* show the highest overall similarity with a value of 0.67 in each cluster. The North American species showed a clustering tendency based on their geographical proximity. *P. juliflora* forms a cluster with four North American species with a similarity index value of 0.425. *P. tamarugo* did not cluster with any other species and remained separate. It is the only species from the section Strombocarpa analysed in the present study. The CC calculated between the original similarity matrix and the cophenetic values from the dendogram (Sokal and Rohlf 1962) shows a value of 0.938. This indicates a higher correlation and lesser distortion between the actual input data matrix and cluster method.

Similarity index

Dice's similarity indices for all the primers are given in table 4. The values ranged from 0 to 0.67. The lowest genetic

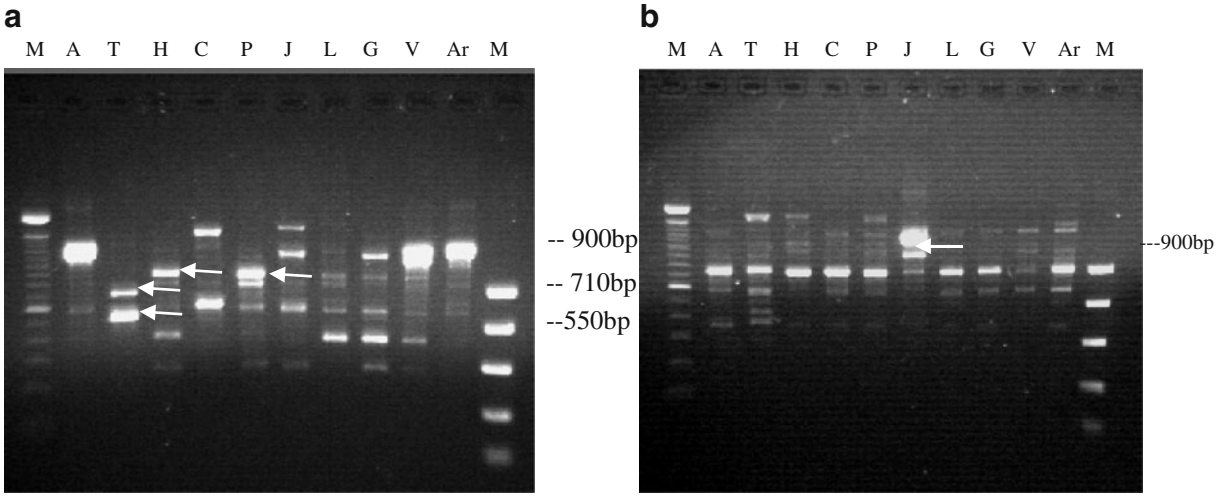

Figure 5. a, RAPD profile of primer N03 and b, R05. For lane names, see footnote of figure 1.

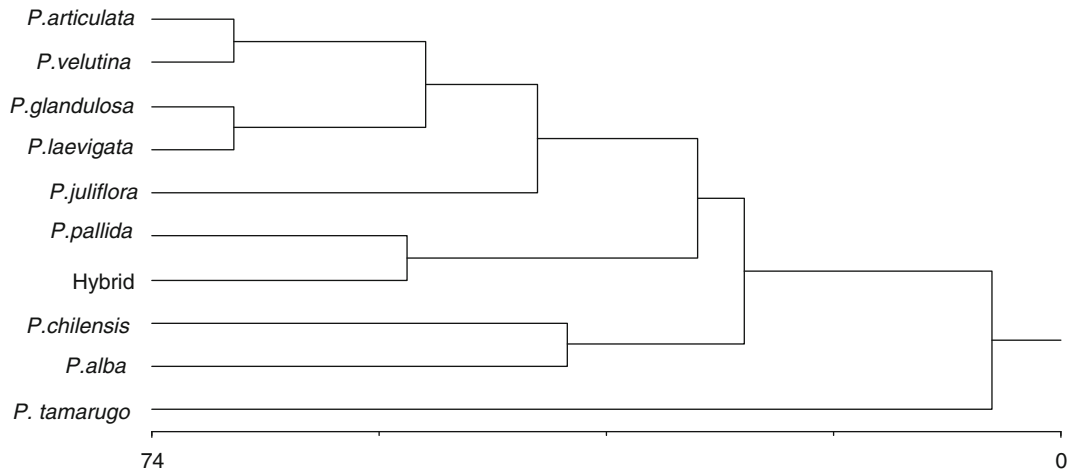

Figure 6. A Group average dendogram of 12 *Prosopis* species based on their RAPD profiles.

similarity was between *P. tamarugo* from section Strombocarpa and *P. articulata* from section Algarobia. *P. tamarugo* is clearly distinct from all the other species. The similarity index of *P. tamarugo* with other species falls below 0.2 which demarcate it from the rest of the species of section Algarobia.

The highest similarity was shown between the North American species, *P. articulata*, *P. velutina*, *P. glandulosa* and *P. laevigata* with an index of 0.67 between them. Similarly, the index between all the North American species selected in the present study is relatively high showing a strong relation in terms of geographical proximity. The distance between *P. pallida* and *P. juliflora* is only 0.27 compared with 0.46 between *P. juliflora* and *P. velutina*. *P. juliflora* appears to be more closely related to the North American species than to the South American *P. pallida* or *P. chilensis*. The hybrid shows closer relation to *P. pallida* than to *P. chilensis* with similarity indices of 0.53 and 0.30, respectively. This data suggests that *P. pallida* is a putative parent of the hybrid.

Cross species amplification of microsatellite markers

Six microsatellite markers developed by Mottura *et al.* (2005) in *P. chilensis* and *P. flexuosa* of section Algarobia were selected for cross species amplification in the genus. These markers were successful in the cross amplification of most of the species belonging to section Algarobia analysed in this study. None of the primer pairs produced amplification in *P. tamarugo* from section Strombocarpa. A summary of the species tested for cross amplification and their amplification pattern is provided in table 5. The amplification product size ranged from approximately 150 bp to 250 bp. The size ranges agreed with the previously published results. The levels of polymorphism among populations were not assessed in this study. Distinctive nonspecific bands with high molecular weight than expected size range were observed with the primer pairs Mo08, Mo09 and Mo16. The percentage transferability of the six primer pairs are provided in table 5. Four out of the six primer pairs showed more than 80% transferability across the 12 *Prosopis* species from two sections of the genus. Of all the primer pairs, Mo08, Mo09 and Mo16 were the most successful in amplifying 12 out of the 13 accessions studied, including the putative *P. pallida* × *P. chilensis* hybrid. The least transferable was the primer pair Mo07.

The primer pair Mo05 with an expected allele size of 218 bp produced bands within the expected size range in most of the species studied (figure 7). Mo05 failed to

Table 4. Similarity index based on RAPD data between 10 *Prosopis* species.

	P. alba	*P. tamarugo*	Hybrid	*P. chilensis*	*P. pallida*	*P. juliflora*	*P. laevigata*	*P. glandulosa*	*P. velutina*	*P. articulata*
P. alba	–									
P. tamarugo	0.03	–								
Hybrid	0.30	0.03	–							
P. chilensis	0.40	0.13	0.30	–						
P. pallida	0.27	0.02	0.53	0.23	–					
P. juliflora	0.18	0.09	0.22	0.18	0.27	–				
P. laevigata	0.17	0.08	0.37	0.21	0.26	0.43	–			
P. glandulosa	0.16	0.09	0.42	0.24	0.27	0.44	0.67	–		
P. velutina	0.32	0.03	0.38	0.30	0.17	0.46	0.53	0.60	–	
P. articulata	0.39	0.00	0.33	0.35	0.26	0.37	0.44	0.49	0.67	–

Table 5. Cross-species amplification in 12 species (one sample per species) from two sections of the genus *Prosopis* using six microsatellite primer pairs developed for *P. chilensis* and *P. flexuosa* (Mottura *et al*. 2005).

Prosopis species	Microsatellite primer pairs					
	Mo05	Mo07	Mo08	Mo09	Mo13	Mo16
P. alba	0	1	1	1	1	1
P. tamarugo	0	0	0	0	0	0
Hybrid	1	0	1	1	0	1
P. chilensis	1	1	1	1	1	1
P. pallida	1	0	1	1	0	1
P. juliflora	1	0	1	1	1	1
P. laevigata	1	1	1	1	1	1
P. glandulosa var. *torreyana*	1	1	1	1	1	1
P. velutina	1	1	1	1	1	1
P. articulata	1	1	1	1	1	1
P. caldenia	1	1	1	1	1	1
P. affinis	1	0	1	1	0	1
P. nigra	1	1	1	1	1	1
Percentage transferability	84.62%	61.54%	92.31%	92.31%	69.23%	92.31%

'1' indicates the presence of fragments in the expected size range, '0' indicates no amplification or unclear bands.

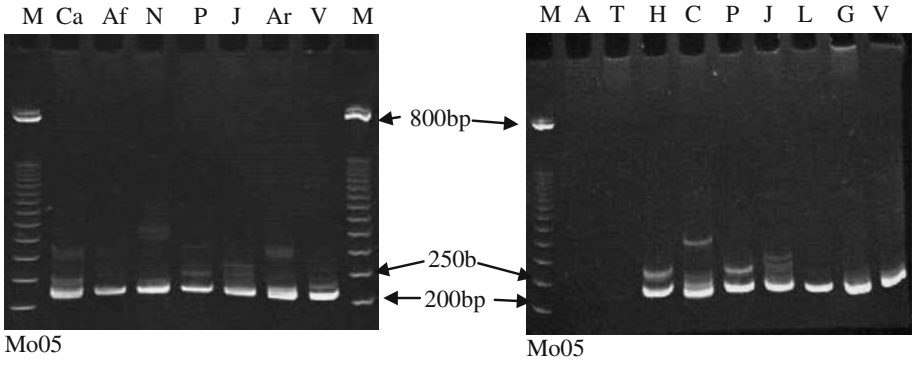

Figure 7. Amplification profile of primer pair Mo05.

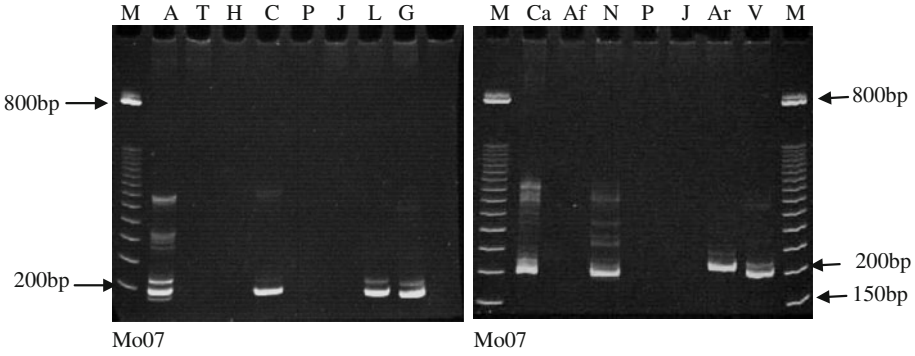

Figure 8. Amplification profile of primer pair Mo07.

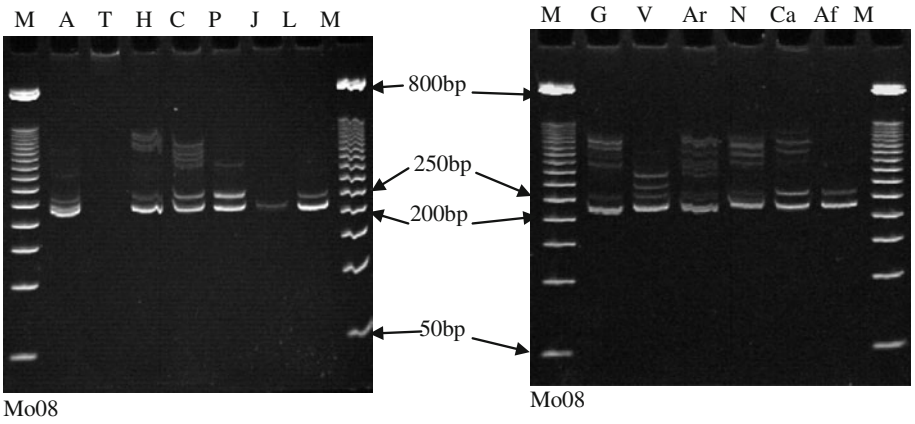

Figure 9. Amplification profile of primer pair Mo08.

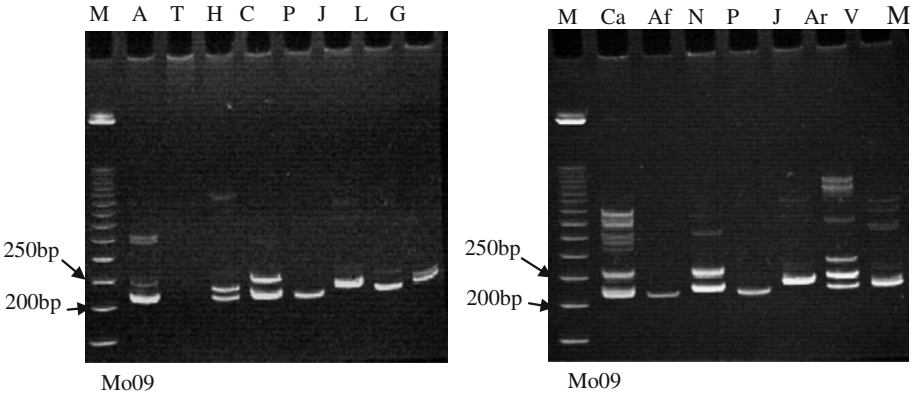

Figure 10. Amplification profile of primer pair Mo09. Lane names- M, molecular size marker; A, *Prosopis alba*; T, *P. tamarugo*; H, putative *P. pallida* × *P. chilensis* hybrid; C, *P. chilensis*; P, *P. pallida*; J, *P. juliflora*; L, *P. laevigata*; G, *P. glandulosa*; Ca, *P. caldenia*; Af, *P. affinis*; N, *P. nigra*; Ar, *P. articulata*; V, *P. velutina.*

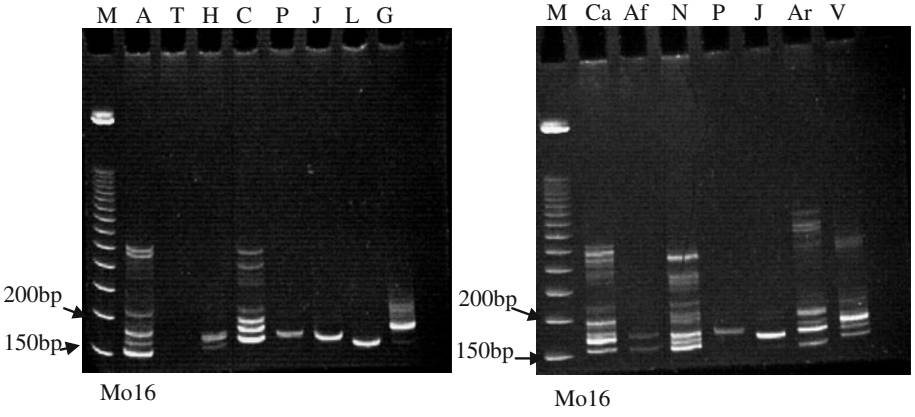

Figure 11. Amplification profile of primer pair Mo16.

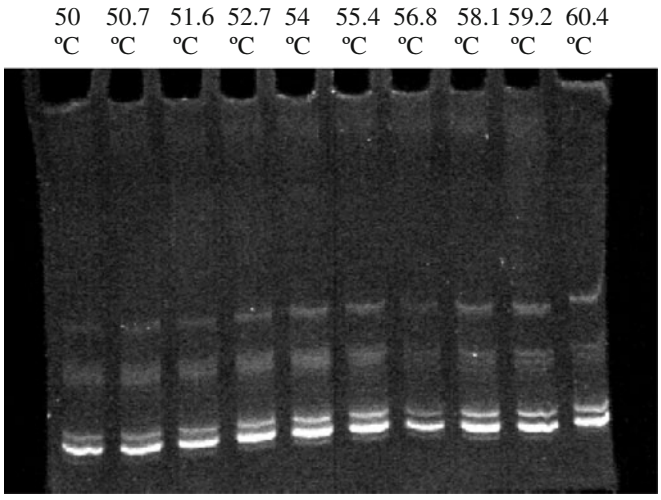

Figure 12. Amplification profile of *P. alba* with microsatellite primer Mo07 observed by varying annealing temperature.

produce amplification in *P. alba* and *P. tamarugo*. Stutter bands could also be seen in some of the species. The primer pairs, Mo07 and Mo13, failed to give amplification in some of the species. The expected sizes of their alleles are 197 bp and 228 bp, respectively. Mo07 produced bands at approximately 200 bp in eight out of the 12 *Prosopis* species studied here (figure 8).

The primer pair Mo08 amplified bands in the expected allele size of 218 bp. It failed to produce any amplification in *P. tamarugo*. Although distinct bands are seen amplified in most of the species, presence of multiple bands and stutter bands are evident (figure 9).

The primer pairs, Mo09 and Mo16, also failed to produce any amplification in *P. tamarugo*. Their expected allele sizes are 233 bp and 157 bp, respectively. Both the primer pairs shows amplification pattern in the expected size range. A number of multiple and stutter bands are also seen in their amplification profile (figures 10 and 11).

An annealing temperature gradient of 50–60.4°C was applied to measure any significant difference in the banding pattern or stuttering. This experiment in *Prosopis alba* has shown that the annealing temperature did not have an effect on the amplification products (figure 12).

Discussion

RAPD (random amplified polymorphic DNA) can detect polymorphisms between genotypes. These are 10-mer arbitrary oligonucleotide primers which can amplify segments of genomic DNA in a variety of species. RAPD markers are dominant and are genotype specific detecting even a single nucleotide difference (Williams *et al.* 1990) and have been successfully used for differentiating species of a genus based on their similarities and geographical proximities (Thomas *et al*. 2001). In the present RAPD analysis, bulked DNA samples were used. Bulking of DNA samples prior to extraction for RAPD analysis will ensure that only the most frequent alleles are favoured during amplification compared to sparse ones (Michelmore *et al.* 1991). Thus the random arbitrary primers target the frequently occurring template regions in the bulked samples, preventing the amplification of rare sequences.

The present work is aimed at analysing the molecular relation between few species in the section Algarobia and to identify any similarity between the tetraploid *P. juliflora* and diploid *P. pallida*. Due to their overlapping geographic range and morphological similarities, *P. juliflora* and *P. pallida* have been grouped as a complex (Pasiecznik *et al.* 2001). Recent works on ploidy by Harris *et al.* (2003) and Trenchard *et al.* (2008) showed that the two species can be distinguished on the basis of their morphology and ploidy. RAPD markers also were useful in differentiating the two species (Landeras *et al.* 2006). However, results obtained suggest that *P. juliflora* is closer to the four North American species identified in this study than to *P. pallida*.

Studies by Landeras *et al.* (2006) have identified RAPD markers which are specific to the *P. Juliflora–P. pallida* complex and also belonging to section Algarobia. These markers could identify the genetic similarity between those species. In the present study, three RAPD primers A08, R18 and S07 yielded markers which were present in all the species belonging to Algarobia but absent in *P. tamarugo* belonging to section Strombocarpa. Ramirez *et al.* (1999) described a similar identifiable marker using the primer S07. The group average clustering method separated *P. tamarugo* from the rest of the *Prosopis* species studied. Sections Strombocarpa and Algarobia are thought to be highly differentiated in terms of speciation process and adaptive strategies (Saidman *et al.* 1996). Studies involving pollen grain morphology (Caccavari De Filice 1972) and foliar architecture (Martinez 1984) and seed protein electrophoresis (Burghardt and Palacios 1997) have clearly differentiated the species of both sections. Moreover morphological and biochemical data involving seven species from Strombocarpa suggest that it constitutes a natural taxon (Burghardt and Espert 2007), unlike Algarobia. Isozyme and amino acid analysis also show that the genetic and morphological differences are high among species in section Strombocarpa compared to Algarobia (Carman *et al*. 1974; Saidman *et al*. 1996). Hence suggestions have been made to treat the two sections as subgenera (Hunziker *et al.* 1986; Saidman *et al.* 1996).

RAPD studies have been useful in identification of interspecific hybrid formation in the genus (Vega and Hernandez 2005). The putative *P. chilensis* × *P. pallida* hybrid and *P. pallida* shared some common bands when amplified with RAPD primers. The RAPD primer S11 yielded similar size markers in *P. pallida* and the hybrid. A less significant marker can also be seen in *P. chilensis*. In their study using RAPD markers, Landeras *et al.* (2006) identified a similar marker using this primer in all the *P. pallida* accessions. These findings suggest that the hybrid is closely

related to *P. pallida*. The faint band present in *P. chilensis* cannot be ruled out suggesting that the hybrid may be an interspecific hybrid between the two species. However, there were not many characteristic bands with other RAPD primers to conclusively identify the mother tree. The oligonucleotide primer S11 is found to be reproducible and hence can be used as a species specific marker for identification of unknown *P. pallida* samples.

The putative mother tree, which fruited in abundance, was identified as *P. chilensis* by Nick Pasiecznik (HDRA, UK). In the vicinity of the mother tree, the only other flowering species found was *P. pallida*. Since *Prosopis* species are selfinfertile, hybridization between the two species was assumed. Hence the sampled seeds were diagnosed as hybrids by the collector (Nick Pasiecznik, personal communication). *P. chilensis* occurs naturally in the arid regions of Peru, Bolivia, central Chile and northwestern Argentina (Johnston 1962; Burkart 1976; National Academy of Sciences 1979). Although the specimen of *P. chilensis* obtained in this study is from Chile, the species is subject to high genetic variability even within populations (Verzino *et al.* 2003). It could be safely concluded that *P. pallida* is one of the parents of the hybrid, though the status of *P. chilensis* could not be confirmed. In their study using RAPD markers, Landeras *et al.* (2006) suggested this possibility of *P. chilensis* as the other putative parent but their results were not conclusive. In the group average cluster analysis in this study, *P. pallida* and the hybrid formed a cluster with a similarity index of 0.582. In the group average dendogram, *P. chilensis* forms a cluster with *P. alba* and their clustering agrees with the result obtained by Ramirez *et al.* (1999).

The highest similarity coefficients in the present study are shown between the North American species, *P. glandulosa* var. *torreyana*, *P. laevigata*, *P. velutina* and *P. articulata*, which range from 0.44 to 0.67. This agrees with the findings of Bessega *et al.* (2000) where isozyme and RAPD studies on populations of *P. laevigata* and *P. glandulosa* proved high genetic similarity between them. Solbrig and Bawa (1975), using isoenzymes found that *P. velutina*, *P. glandulosa* var. *glandulosa* and *P. leavigata* are closely related. The *P. juliflora* sample from the Galapagos Islands shows great affinity to the North American species. The clustering pattern clearly shows that the genetic similarity between *P. juliflora* and the North American species analysed here is higher than the similarity expected with *P. pallida*.

Two of the North American species selected here, *P. glandulosa* and *P. laevigata*, are from their native ranges. The outcrossing nature of *Prosopis* species could contribute genetic variability among them. Hence the clustering pattern observed among introduced North American species studied here should be treated with caution. The similarity coefficients between *P. juliflora* and North American species ranges from 0.37 to 0.67, which is slightly lesser than the values previously reported (Juárez-Muñoz *et al.* 2006). Their study also suggests that *P. laevigata* and *P. glandulosa* are related with a similarity index of 0.79. However the similarity index values between *P. glandulosa* and *P. velutina* is considerably less than the value of 0.60 obtained in this study. Enzyme marker studies on *Prosopis* by Bessega *et al.* (2005), suggest that *P. velutina* and *P. glandulosa* are so genetically distinct that they would have originated from two independent founder events.

Present work on RAPD markers proved to be helpful in clarifying the identity of the *Prosopis* species selected in this study. The origin of tetraploid *P. juliflora* still remains unanswered but this study has attempted to solve some of the questions regarding its relation to *P. pallida*. It is not known whether *P. julilfora* is an allotetraploid involving *P. pallida* or an autotetraploid. The *P. juliflora* sample from the Galapagos Islands could possess some variation with respect to the ones from the rest of the native range, but the evidence provided seems valid enough to conclude that the two species are genetically dissimilar. The differentiation of *P. pallida* and *P. juliflora* based on ploidy and morphology (Harris *et al.* 2003; Trenchard *et al.* 2008) corresponds with the RAPD data obtained in the present study, although more accessions of each species from their native ranges would give a more conclusive result. The clustering pattern of the *Prosopis* species selected in this work does not agree with the morphological criteria proposed by Burkart (1976), since species of the same series are not clustered together. There seems to be incongruence between the morphological and molecular data in the case of *Prosopis* species analysed here, and similar inconsistency was also observed in previous studies by many authors (Saidman and Vilardi 1987; Bessega *et al.* 2005, 2006).

A total of six previously published microsatellite primer pairs developed in *P. chilensis* and *P. flexuosa* were tested for transferability in 12 *Prosopis* species. Except for *P. tamarugo*, all the other species are from section Algarobia of the genus. All the six microsatellite primer pairs were transferable in most of the Algarobian species, but failed to produce a single amplification in *P. tamarugo.* Mottura *et al.* (2005) tested these six primer pairs for cross-species amplification in seven species of *Prosopis* from the two sections Algarobia and Strombocrapa. Nine additional species including *P. tamarugo* were included in the present transferability study compared to Mottura *et al.* (2005). In their study, at least three primer pairs gave amplification in all the species tested, but the rate of amplification was comparatively less in Strombocarpa than in Algarobia. Based on the present evidence on microsatellite transferability it cannot be concluded that these markers could be used to differentiate between different sections of the genus. However, the failure of amplification could be due to lack of any conserved primer binding sites in the DNA for amplification to occur (Weising *et al.* 2005). Any intraspecific sequence variation in the primer flanking sites also could interfere with primer binding (Angers and Bernatchez 1997; Colson and Goldstein 1999). In a study by Butcher *et al.* (2000), the microsatellite primers developed in *A. mangium* failed to show any amplification in species from a different subgenus but produced high rates

of amplification in species of the same section. Transferability of SSR markers were also observed among distant taxa (Yasodha *et al*. 2005).

In some species, an intense band can be seen with relatively less intense ones above it. Although there are stutter bands or multiple bands present in each locus, the microsatellite markers were consistent in amplification under the same PCR conditions and all of the bands are scorable. The nonspecific bands observed in some of the species at the microsatellite loci is likely due to the primer mismatch positions from the *Prosopis* species DNA analysed here. Formation of stutter bands or multiple bands is common while amplifying SSR markers between species. This can be reduced by changing the annealing temperature (Rossetto 2001). However, change in annealing temperature did not vary the banding profile observed with *P. alba* in the present study. These bands could interfere with the targeted locus and completely or partially prevent their amplification (Primmer *et al.* 1996). Cloning experiments have shown that these nonspecific or stutter bands have one or few repeat units missing (Luty *et al.* 1990).

The high percentage of transferability of primers across *Prosopis* species indicates high level of sequence similarity in the DNA sequences flanking microsatellites. Four out of six primers gave a transferability percentage of more than 80% and showed robustness in subsequent amplification reactions. The higher cross amplification rate of microsatellites observed in this study would help in the genetic evaluation of a broad range of species in this genus and would provide information regarding their mating system and gene flow.

To conclude, RAPD and microsatellite transferability studies in these *Prosopis* species proved helpful in clearly demarcating the species belonging to two different sections, Algarobia and Strombocarpa. Perhaps analysis of more species from Strombocarpa could confirm the separation. Moreover the relationship between different species of section Algarobia has also been identified through this study. The tetraploid *P. juliflora* and diploid *P. pallida* samples analysed in this study did not seem to be genetically related. This preliminary study suggests that within section Algarobia the grouping of series seems artificial, agreeing with many other studies, though the fact that a few of the *Prosopis* species are not from their native range should also be considered while interpreting the results, as also that *Prosopis* species are susceptible to genetic variation due to their protogynous nature, out breeding and environmental factors. Molecular marker analysis of a wide range of species from their native range and the many sections could help to resolve the taxonomic confusion surrounding this genus and would help in the proper exploitation of economically important species.

Acknowledgement

This research is a part of self funded Ph.D. course undertaken at Coventry University. We would like to thank anonymous reviewers for their valuable suggestions and thorough review of this work.

References

Angers B. and Bernatchez L. 1997 Complex evolution of a salmonid microsatellite locus and its consequences in inferring allelic divergence from size information. *Mol. Biol. Evol.* **14**, 230–238.

Bessega C., Ferreyra L., Julio N., Montoya S., Saidman B. and Vilardi J. C. 2000 Mating system parameters in species of genus *Prosopis* (Leguminosae). *Hereditas* **132**, 19–27.

Bessega C., Saidman B. O. and Vilardi J. C. 2005 Genetic relationships among American species of *Prosopis* (Leguminosae) based on enzyme markers. *Genet. Mol. Biol.* **28**, 277–286.

Bessega C., Vilardi J. C. and Saidman B. O. 2006 Genetic relationships among American species of the genus *Prosopis* (Mimosoideae, Leguminosae) inferred from ITS sequences: evidence for long term dispersal. *J. Biogeogr.* **33**, 1905–1915.

Burghardt A. D. and Palacios R. A. 1997 Electrophoretic characterization of the American sections of *Prosopis* L. (Leguminosae: Mimosoidae). *Bull. Int. Group study Mimosoideae* **20**, 71–83.

Burghardt A. D. and Espert S. M. 2007 Phylogeny of *Prosopis* (Leguminosae) as shown by morphological and biochemical evidence. *Aust. Syst. Bot.* **20**, 332–339.

Burkart A. 1976 A Monograph of the genus *Prosopis* (Leguminosae subfam. Mimosoideae). (Part 1& 2). Catalogue of the recognised species of *Prosopis. J. Arnold Arbor.* **57**, 219–249 (part 1) and **57**, 450–525 (part 2).

Butcher P. A., Decroocq S., Gray Y. and Moran G. F. 2000 Development, inheritance and cross-species amplification of microsatellite markers from *Acacia mangium*. *Theor. Appl. Genet.* **101**, 1282–1290.

Caccavari De Filice M. A. 1972 Granos de polen de leguminosas de la Argentina II. subfam. mimosoideae; tribu Adenanthereae. *Revta. Mus. Argent. Cienc. Nat. Bernardino Rivadavia, Bol*. **4**, 281–320.

Carman N. J., Dossaji S. F. and Mabry T. J. 1974 A populational survey of amino acids in *Prosopis* species from North and South America. *Biochem. Syst. Ecol.* **2**, 73–74.

Colson I. and Goldstein D. B. 1999 Evidence for complex mutations at microsatellite loci in *Drosophila*. *Genetics* **152**, 617–627.

Dice L. R. 1945 Measures of the amount of ecologic association between species. *Ecology* **26**, 297–302.

Everitt B. and Dunn G. 2001 *Applied multivariate data analysis*, 2nd edition. Arnold Publishers, London, UK.

Ferreyra L. I., Bessega C., Vilardi J. C. and Saidman B. O. 2007 Consistency of population genetics parameters estimated from isozyme and RAPDs dataset in species of genus *Prosopis* (Leguminosae, Mimosoideae). *Genetica* **131**, 217–230.

Goswami M. and Ranade S. A. 1999 Analysis of variations in RAPD profiles among accessions of *Prosopis. J. Genet.* **78**, 141–147.

Hair J. P., Black W. C., Babin B. J., Anderson R. E. and Tatham R. L. 2006 *Multivariate data analysis*, 6th edition. Prentice Hall, New Jersey, USA.

Harris P. J. C., Pasiecznik N. M., Smith S. J., Billington J. M. and Ramirez L. 2003 Differentiation of *Prosopis juliflora* (Sw.) DC & *Prosopis pallida* (H.&.B. ex Willd.) H.B.K. using foliar characters and ploidy. *For. Ecol. Manag.* **180**, 153–164.

Hughes C. E. 1994 Risks of species introductions in tropical forestry. *Commonw. For. Rev.* **73**, 243–252.

Hunziker J. H., Saidman B. O., Naranjo C. A., Palacios R. A., Poggio L. and Burghardt A. D. 1986 Hybridization and genetic variation of Argentine species of *Prosopis*. *For. Ecol. Manag.* **16**, 301–315.

Johnston M. C. 1962 The North American Mesquites *Prosopis* Sect. Algarobia (Leguminosae). *Brittonia* **14**, 72–90.

Juárez-Muñoz J., Carrillo-Castañeda G. and Rubluo A. 2006 Polymorphism determination in two natural mezquite (*Prosopis*

laevigata) populations using RAPD. *Biotecnol. Apl.* **23**, 229–235.

Kent M. and Coker P. 1992 *Vegetation description and analysis: a practical approach*. Belhaven Press, London, UK.

Landeras G., Alfonso M., Pasiecznik N. M., Harris P. J. C. and Ramirez L. 2006 Identification of *Prosopis juliflora* and *Prosopis pallida* accessions using molecular markers. *Biodiversity Conserv*. **15**, 1829–1844.

Lessig V. P. 1972 Comparing cluster analyses with cophenetic correlation. *J. Mark. Res.* **9**, 82–84.

Luty J. A., Guo Z., Willard H. F., Ledbetter D. H., Ledbetter S. and Litt M. 1990 Five polymorphic microsatelleite VNTRs on the human X chromosome. *Am. J. Hum. Genet.* **46**, 776–783.

Manly B. F. J. 2005 *Multivariate statistical methods, a primer*, 3rd edition. Chapman Hall, London, UK.

Mantel N. A. 1967 The detection of disease clustering and a generalized regression approach. *Cancer Res.* **27**, 209–220.

Martinez S. 1984 Foliar architecture of species of the genus *Prosopis*. *Darwiniana* **25**, 279–297.

Michelmore R. W., Paran I. and Kesseli R. V. 1991 Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. *Proc. Natl. Acad. Sci. USA* **88**, 9828–9832.

Mottura M., Finkeldey R., Verga A. and Gailing O. 2005 Development and characterization of microsatellite markers for *Prosopis chilensis* and *Prosopis flexuosa* and cross-species amplification. *Mol. Ecol. Notes* **5**, 487–489.

Naranjo C. A, Poggio L. and Zieger S. E. 1984 Phenol chromatography, morphology and cytogenetics in 3 species and natural hybrids of *Prosopis* (Leguminosae-mimosoideae). *Plant Syst. Evol.* **144**, 257–276.

National Academy of Sciences 1979 *Tropical legumes: resources for the future*. National Academy Press, Washington DC, USA.

Pasiecznik N. M., Felker P., Harris P. J. C., Harsh L. N., Cruz G., Tewari J. C. *et al.* 2001 *The Prosopis juliflora-Prosopis pallida complex: a monograph*. Henry Doubleday Research Association, Coventry.

Pasiecznik N. M., Harris P. J. C. and Smith S. J. 2004 *Identifying tropical Prosopis species: a field guide*. Henry Doubleday Research Association, Coventry.

Primmer C. and Merila J. 2002 A low rate of cross-species microsatellite amplification success in Ranid frogs. *Conserv. Genet.* **3**, 445–449.

Primmer C. R., Moller A. P. and Ellegren H. 1996 A wide-range survey of cross-species microsatellite amplification in birds. *Mol. Ecol.* **5**, 365–378.

Ramirez L., De la Vega A., Razkin N., Luna V. and Harris P. J. C. 1999 Analysis of the relationship between species of the Genus *Prosopis* revealed by the use of molecular markers. *Agronomie* **19**, 31–43.

Romesberg H. C. 1984 *Cluster analysis for researchers*. Lifetime Learning Publications, Belmont, USA.

Romesberg H. C. 2004 Cluster analysis for researchers, Lulu Press, North Carolina.

Rosseto M., McNally J. and Henry R. J. 2002 Evaluating the potential of SSR flanking regions for examining taxonomic relationships in the Vitaceae. *Theor. Appl. Genet*. **104**, 61–66.

Rossetto M. 2001 Sourcing of SSR Markers from related plant species. In *Plant genotyping: the DNA fingerprinting of plants* (ed. R. J. Henry), pp. 210–224. CAB International, Oxford, UK.

Saidman B. O. and Vilardi J. C. 1987 Analysis of the genetic similarities among seven species of *Prosopis* (Leguminosae: Mimosoideae). *Theor. Appl. Genet.* **75**, 109–116.

Saidman B. O., Vilardi J. C., Pocovi M. I. and Acreche N. 1996 Genetic divergence among species of the section Strombocarpa genus *Prosopis* (Leguminosae). *J. Genet.* **75**, 139–149.

Simpson B. B. 1977 Breeding system of dominant perennial plants of two disjunct warm desert ecosystems. *Oecologia* **27**, 203–226.

Sokal R. R. and Michener C. D. 1958 A Statistical method for evaluating systematic relationships. *Univ. Kans. Sci. Bull.* **38**, 1409–1438.

Sokal R. R. and Rohlf F. J. 1962 The comparison of dendrograms by objective methods. *Taxon* **11**, 33–40.

Solbrig O. T. and Bawa K. S. 1975 Isozyme variation in species of *Prosopis* (Leguminosae). *J. Arnold Arbor.* **56**, 398–826.

Solbrig O. T. and Cantino P. D. 1975 Reproductive adaptations in *Prosopis* (Leguminosae, Mimosoideae). *J. Arnold Arbor.* **56**, 185–210.

Thomas G., Sreejayan., Joseph L. and Kuriachen P. 2001 Genetic variation and population structure in *Oryza malampuzhaensis* Krish. et Chand. endemic to Western Ghats, South India. *J. Genet.* **80**, 141–148.

Trenchard L. J., Harris P. J. C., Smith S. J. and Pasiecznik N. M. 2008 A review of ploidy in the genus *Prosopis* (Leguminosae). *Bot. J. Linn. Soc. Suppl.* **156**, 425–438.

Vega M. V. and Hernandez P. 2005 Molecular evidence for natural interspecific hybridisation in *Prosopis*. *Agrofor. Syst.* **64**, 197–202.

Verzino G., Carrnza C., Ledesma M., Joueaui J. and Dirienzo J. 2003 Adaptive genetic variation of *Prosopis chilensis* (Mol) Stuntz, preliminary results from one test-site. *For. Ecol. Manag.* **175**, 119–129.

Weising K., Nybom H., Wolff K. and Kahl G. 2005 *DNA fingerprinting in plants: principles, methods and applications*, 2nd edition, pp. 180–183. CRC Press, Boca Raton, USA.

Whitmore D. H. and Bragg L. H. 1979 Isozymal differentiation between two species of *Prosopis. Biochem. Syst. Ecol.* **7**, 299–302.

Wiggins I. and Porter D. 1971 *Flora of the Galapagos Islands*. Stanford University, Stanford, USA.

Williams J. G. K., Kubelik A. R., Livak K. J., Rafalski J. A. and Tingey S. V. 1990 DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*. **18**, 6531–6535.

Yasodha R., Ghosh M., Sumathi R. and Gurumurthi K. 2005 Cross-species amplification of eucalyptus SSR markers in Casuarinaceae. *Acta Bot. Croat.* **64**, 115–120.

Received 30 September 2010; accepted 11 February 2011
Published on the Web: 19 August 2011